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OIPE

## RAW SEQUENCE LISTING

DATE: 10/19/2001

PATENT APPLICATION: US/09/878,131

TIME: 10:50:38

Input Set : A:\Lee113.app

Output Set: N:\CRF3\10192001\I878131.raw

ENTERED

p.5

3 <110> APPLICANT: FAN HONG, GUO  
 4 YANG, YONGJIE  
 5 ZHU, JIA  
 7 <120> TITLE OF INVENTION: LOW TEMPERATURE CYCLE EXTENSION OF DNA WITH HIGH  
 8 POLYMERIZATION SPECIFICITY  
 10 <130> FILE REFERENCE: LEE 113  
 12 <140> CURRENT APPLICATION NUMBER: 09/878,131  
 13 <141> CURRENT FILING DATE: 2001-06-08  
 15 <160> NUMBER OF SEQ ID NOS: 21  
 17 <170> SOFTWARE: PatentIn Ver. 2.1  
 19 <210> SEQ ID NO: 1  
 20 <211> LENGTH: 1764  
 21 <212> TYPE: DNA  
 22 <213> ORGANISM: Bacillus stearothermophilus  
 24 <400> SEQUENCE: 1

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 26 gagatgcttg ccgacaaggc agcgcttgct gttgaggtga tggaagaaaa ctaccacgat 120  
 27 gccccgattg tcggaatcgc actagtgaac gagcatgggc gattttttat gcgcccggag 180  
 28 accgcgctgg ctgattcgca atttttagca tggcttgccg atgaaacgaa gaaaaaaagc 240  
 29 atgtttgacg ccaagcgggc agtcgttgcc ttaaagtgga aaggaattga gcttcgcggc 300  
 30 gtcgcctttg atttattgct cgctgcctat ttgctcaatc cggctcaaga tgccggcgat 360  
 31 atcgctgcgg tggcgaaaat gaaacaatat gaagcgggtg ggtcggatga agcgggtctat 420  
 32 ggcaaaggcg tcaagcggtc gctgccggac gaacagacgc ttgctgagca tctcgttcgc 480  
 33 aaagcggcag ccatttgggc gcttgagcag ccgtttatgg acgatttgcg gaacaacgaa 540  
 34 caagatcaat tattaacgaa gcttgagcac gcgctggcgg cgattttggc tgaaatggaa 600  
 35 ttacttgggg tgaacgtgga tacaagcgg cttgaacaga tgggttcgga gctcgcgcaa 660  
 36 caactgcgtg ccacgagca gcgcatttac gagctagccg gccaaagagt caacattaac 720  
 37 tcaccaaacc agctcggagt cattttatgt gaaaagctgc agctaccggt gctgaagaag 780  
 38 acgaaaacag gctattcgac ttcggctgat gtgcttgaga agcttgcgcc gcatcatgaa 840  
 39 atcgctgaaa acattttgca ttaccgccag cttggcaaac tgcaatcaac gtatattgaa 900  
 40 ggattgttga aagttgtgcy ccctgatacc ggcaaagtgc atacgatgtt caaccaagcg 960  
 41 ctgacgcaaa ctgggcggct cagctcggcc gagccgaact tgcaaaacat tccgattcgg 1020  
 42 ctggaagagg ggcggaaaat ccgccaagcg ttcgtcccgt cagagccgga ctggctcatt 1080  
 43 ttcgcccggc attactcaca aattgaattg cgcgtcctcg cccatatcgc cgatgacgac 1140  
 44 aatctaattg aagcgttcca acgcgatttg gatattcaca caaaaacggc gatggacatt 1200  
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 46 ttcggtatcg tttacggaat tagcgattac ggattggcgc aaaacttgaa cattacgcgc 1320  
 47 aaagaagctg ccgaatttat cgaacgttac ttcgccagct ttcggggcgt aaagcagtat 1380  
 48 atggaaaaca tagtgcaaga agcgaaacag aaaggatatg tgacaacgct gttgcatcgg 1440  
 49 cgccgctatt tgccgtgatat tacaagccgc aatttcaacg tccgcagttt tgcagagcgg 1500  
 50 acggccatga acacgccaat tcaagggaag gccgctgaca ttattaaaaa agcagtgatt 1560  
 51 gatttagcgg cacggctgaa agaagagcag cttcaggctc gtcttttgct gcaagtgcat 1620  
 52 gacgagctca ttttggaagc gccaaaagag gaaattgagc gattatgtga gcttggtccg 1680  
 53 gaagtgatgg agcaggccgt tacgctccgc gtgccgctga aagtcgacta ccattacggc 1740  
 54 ccaacatggt atgatgccaa ataa 1764  
 57 <210> SEQ ID NO: 2  
 58 <211> LENGTH: 586

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59 <212> TYPE: PRT
60 <213> ORGANISM: Bacillus stearothermophilus
62 <400> SEQUENCE: 2
63 Ala Glu Gly Glu Lys Pro Leu Glu Glu Met Glu Phe Ala Ile Val Asp
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66 Val Ile Thr Glu Glu Met Leu Ala Asp Lys Ala Ala Leu Val Val Glu
67           20           25           30
69 Val Met Glu Glu Asn Tyr His Asp Ala Pro Ile Val Gly Ile Ala Leu
70           35           40           45
72 Val Asn Glu His Gly Arg Phe Phe Met Arg Pro Glu Thr Ala Leu Ala
73           50           55           60
75 Asp Ser Gln Phe Leu Ala Trp Leu Ala Asp Glu Thr Lys Lys Lys Ser
76   65           70           75           80
78 Met Phe Asp Ala Lys Arg Ala Val Val Ala Leu Lys Trp Lys Gly Ile
79           85           90           95
81 Glu Leu Arg Gly Val Ala Phe Asp Leu Leu Leu Ala Ala Tyr Leu Leu
82           100          105          110
84 Asn Pro Ala Gln Asp Ala Gly Asp Ile Ala Ala Val Ala Lys Met Lys
85           115          120          125
87 Gln Tyr Glu Ala Val Arg Ser Asp Glu Ala Val Tyr Gly Lys Gly Val
88           130          135          140
90 Lys Arg Ser Leu Pro Asp Glu Gln Thr Leu Ala Glu His Leu Val Arg
91   145          150          155          160
93 Lys Ala Ala Ala Ile Trp Ala Leu Glu Gln Pro Phe Met Asp Asp Leu
94           165          170          175
96 Arg Asn Asn Glu Gln Asp Gln Leu Leu Thr Lys Leu Glu His Ala Leu
97           180          185          190
99 Ala Ala Ile Leu Ala Glu Met Glu Phe Thr Gly Val Asn Val Asp Thr
100          195          200          205
102 Lys Arg Leu Glu Gln Met Gly Ser Glu Leu Ala Glu Gln Leu Arg Ala
103          210          215          220
105 Ile Glu Gln Arg Ile Tyr Glu Leu Ala Gly Gln Glu Phe Asn Ile Asn
106   225          230          235          240
108 Ser Pro Lys Gln Leu Gly Val Ile Leu Phe Glu Lys Leu Gln Leu Pro
109          245          250          255
111 Val Leu Lys Lys Thr Lys Thr Gly Tyr Ser Thr Ser Ala Asp Val Leu
112          260          265          270
114 Glu Lys Leu Ala Pro His His Glu Ile Val Glu Asn Ile Leu His Tyr
115          275          280          285
117 Arg Gln Leu Gly Lys Leu Gln Ser Thr Tyr Ile Glu Gly Leu Leu Lys
118          290          295          300
120 Val Val Arg Pro Asp Thr Lys Val His Thr Met Phe Asn Gln Ala Leu
121   305          310          315          320
123 Thr Gln Thr Gly Arg Leu Ser Ser Ala Glu Pro Asn Leu Gln Asn Ile
124          325          330          335
126 Pro Ile Arg Leu Glu Glu Gly Arg Lys Ile Arg Gln Ala Phe Val Pro
127          340          345          350
129 Ser Glu Pro Asp Trp Leu Ile Phe Ala Ala Asp Tyr Ser Gln Ile Glu
130          355          360          365

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132 Leu Arg Val Leu Ala His Ile Ala Asp Asp Asp Asn Leu Ile Glu Ala
133      370                      375                      380
135 Phe Gln Arg Asp Leu Asp Ile His Thr Lys Thr Ala Met Asp Ile Phe
136 385                      390                      395                      400
138 Gln Leu Ser Glu Glu Glu Val Thr Ala Asn Met Arg Arg Gln Ala Lys
139                      405                      410                      415
141 Ala Val Asn Phe Gly Ile Val Tyr Gly Ile Ser Asp Tyr Gly Leu Ala
142                      420                      425                      430
144 Gln Asn Leu Asn Ile Thr Arg Lys Glu Ala Ala Glu Phe Ile Glu Arg
145                      435                      440                      445
147 Tyr Phe Ala Ser Phe Pro Gly Val Lys Gln Tyr Met Glu Asn Ile Val
148                      450                      455                      460
150 Gln Glu Ala Lys Gln Lys Gly Tyr Val Thr Thr Leu Leu His Arg Arg
151 465                      470                      475                      480
153 Arg Tyr Leu Pro Asp Ile Thr Ser Arg Asn Phe Asn Val Arg Ser Phe
154                      485                      490                      495
156 Ala Glu Arg Thr Ala Met Asn Thr Pro Ile Gln Gly Ser Ala Ala Asp
157                      500                      505                      510
159 Ile Ile Lys Lys Ala Met Ile Asp Leu Ala Ala Arg Leu Lys Glu Glu
160                      515                      520                      525
162 Gln Leu Gln Ala Arg Leu Leu Leu Gln Val His Asp Glu Leu Ile Leu
163                      530                      535                      540
165 Glu Ala Pro Lys Glu Glu Ile Glu Arg Leu Cys Glu Leu Val Pro Glu
166 545                      550                      555                      560
168 Val Met Glu Gln Ala Val Thr Leu Arg Val Pro Leu Lys Val Asp Tyr
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171 His Tyr Gly Pro Thr Trp Tyr Asp Ala Lys
172                      580                      585
175 <210> SEQ ID NO: 3
176 <211> LENGTH: 1764
177 <212> TYPE: DNA
178 <213> ORGANISM: Artificial Sequence ✓
180 <220> FEATURE:
181 <223> OTHER INFORMATION: Description of Artificial Sequence: Modified Bst ✓
182      DNA sequence
184 <400> SEQUENCE: 3
185 atggccgaag gggagaaacc gcttgaggag atggagtttg ccatcggtga cgtcattacc 60
186 gaagagatgc ttgccgacaa ggcagcgctt gtcgttgagg tgatggaaga aaactaccac 120
187 gatgccccga ttgtcggaat cgcactagtg aacgagcatg ggcgattttt tatgcgcccg 180
188 gagaccgcgc tggctgattc gcaattttta gcatggcttg ccgatgaaac gaagaaaaaa 240
189 agcatgtttg acgccaagcg ggcagtcggt gccttaaagt ggaaaggaat tgagcttcgc 300
190 ggcgtcgctt ttgatttatt gctcgctgcc tatttgctca atccggctca agatgccggc 360
191 gatatcgctg cgggtggcgaa aatgaaacaa tatgaagcgg tgcggtcgga tgaagcggtc 420
192 tatggcaaaag gcgtaagcg gtcgctgccg gacgaacaga cgcttgctga gcatctcggt 480
193 cgcaaagcgg cagccatttg ggcgcttgag cagccgttta tggacgattt gcggaacaac 540
194 gaacaagatc aattattaac gaagcttgag cacgcgctgg cggcgatttt ggctgaaatg 600
195 gaattcactg gggatgaacg ggatacaaa cggcttgaa agatgggttc ggagctcgcc 660
196 gaacaactgc gtgccatcga gcagcgcat tacgagctag ccggccaaga gttcaacatt 720
197 aactcaccaa aacagctcgg agtcatttta tttgaaaagc tgcagctacc ggtgctgaag 780

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198 aagacgaaaa caggctattc gacttcgggt gatgtgcttg agaagcttgc gccgcatcat 840
199 gaaatcgctg aaaacatttt gcattaccgc cagcttgcca aactgcaatc aacgtatatt 900
200 gaaggattgt tgaaagttgt gcgccctgat accggcaaaag tgcatacgat gttcaaccaa 960
201 gcgctgacgc aaactgggcg gctcagctcg gccgagccga acttgcaaaa cattccgatt 1020
202 cggacccccac tggggcgcaa aatccgccaa gcgttcgtcc cgtcagagcc ggactggctc 1080
203 attttcgccg ccgattactc acaaattgaa ttgcgcgtcc tcgcccatat cgccgatgac 1140
204 gacaatctaa ttgaagcgtt ccaacgcgat ttggatattc acacaaaaac ggcgatggac 1200
205 attttccagt tgagcgaaga ggaagtcacg gccaacatgc gccgccaggc aaaggccggt 1260
206 aactacggta tcgtttacgg aattagcgat tacggattgg cgcaaaactt gaacattacg 1320
207 cgcaaagaag ctgccgaatt tatcgaacgt tacttcgcca gctttccggg cgtaaagcag 1380
208 tatatggaaa acatagtga agaagcgaaa cagaaaggat atgtgacaac gctgtgcat 1440
209 cggcgccgct atttgcctga tattacaagc cgcaatttca acgtccgcag ttttgagag 1500
210 cggacggcca tgaacacgcc aattcaagga agcgccgctg acattattaa aaaagcgatg 1560
211 attgatttag cggcacggct gaaagaagag cagcttcagg ctcgtctttt gctgcaagt 1620
212 catgacgagc tcatttttga agcgccaaaa gaggaattg agcgattatg tgagcttggt 1680
213 ccggaagtga tggagcaggc cgttacgctc cgcggtgccg tgaaagtcga ctaccattac 1740
214 ggcccaacat ggtatgatgc caaa 1764

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217 &lt;210&gt; SEQ ID NO: 4

218 &lt;211&gt; LENGTH: 588

219 &lt;212&gt; TYPE: PRT

220 &lt;213&gt; ORGANISM: Artificial Sequence

222 &lt;220&gt; FEATURE:

223 &lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence: Modified Bst

224 amino acid sequence

226 &lt;400&gt; SEQUENCE: 4

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227 Met Ala Glu Gly Glu Lys Pro Leu Glu Glu Met Glu Phe Ala Ile Val
228 1 5 10 15
230 Asp Val Ile Thr Glu Glu Met Leu Ala Asp Lys Ala Ala Leu Val Val
231 20 25 30
233 Glu Val Met Glu Glu Asn Tyr His Asp Ala Pro Ile Val Gly Ile Ala
234 35 40 45
236 Leu Val Asn Glu His Gly Arg Phe Phe Met Arg Pro Glu Thr Ala Leu
237 50 55 60
239 Ala Asp Ser Gln Phe Leu Ala Trp Leu Ala Asp Glu Thr Lys Lys Lys
240 65 70 75 80
242 Ser Met Phe Asp Ala Lys Arg Ala Val Val Ala Leu Lys Trp Lys Gly
243 85 90 95
245 Ile Glu Leu Arg Gly Val Ala Phe Asp Leu Leu Leu Ala Ala Tyr Leu
246 100 105 110
248 Leu Asn Pro Ala Gln Asp Ala Gly Asp Ile Ala Ala Val Ala Lys Met
249 115 120 125
251 Lys Gln Tyr Glu Ala Val Arg Ser Asp Glu Ala Val Tyr Gly Lys Gly
252 130 135 140
254 Val Lys Arg Ser Leu Pro Asp Glu Gln Thr Leu Ala Glu His Leu Val
255 145 150 155 160
257 Arg Lys Ala Ala Ala Ile Trp Ala Leu Glu Gln Pro Phe Met Asp Asp
258 165 170 175
260 Leu Arg Asn Asn Glu Gln Asp Gln Leu Thr Lys Leu Glu His Ala
261 180 185 190

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TIME: 10:50:38

Input Set : A:\Leel13.app

Output Set: N:\CRF3\10192001\I878131.raw

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263 Leu Ala Ala Ile Leu Ala Glu Met Glu Phe Thr Gly Val Asn Val Asp
264      195      200      205
266 Thr Lys Arg Leu Glu Gln Met Gly Ser Glu Leu Ala Glu Gln Leu Arg
267      210      215      220
269 Ala Ile Glu Gln Arg Ile Tyr Glu Leu Ala Gly Gln Glu Phe Asn Ile
270 225      230      235      240
272 Asn Ser Pro Lys Gln Leu Gly Val Ile Leu Phe Glu Lys Leu Gln Leu
273      245      250      255
275 Pro Val Leu Lys Lys Thr Lys Thr Gly Tyr Ser Thr Ser Ala Asp Val
276      260      265      270
278 Leu Glu Lys Leu Ala Pro His His Glu Ile Val Glu Asn Ile Leu His
279      275      280      285
281 Tyr Arg Gln Leu Gly Lys Leu Gln Ser Thr Tyr Ile Glu Gly Leu Leu
282      290      295      300
284 Lys Val Val Arg Pro Asp Thr Gly Lys Val His Thr Met Phe Asn Gln
285 305      310      315      320
287 Ala Leu Thr Gln Thr Gly Arg Leu Ser Ser Ala Glu Pro Asn Leu Gln
288      325      330      335
290 Asn Ile Pro Ile Arg Thr Pro Leu Gly Arg Lys Ile Arg Gln Ala Phe
291      340      345      350
293 Val Pro Ser Glu Pro Asp Trp Leu Ile Phe Ala Ala Asp Tyr Ser Gln
294      355      360      365
296 Ile Glu Leu Arg Val Leu Ala His Ile Ala Asp Asp Asp Asn Leu Ile
297      370      375      380
299 Glu Ala Phe Gln Arg Asp Leu Asp Ile His Thr Lys Thr Ala Met Asp
300 385      390      395      400
302 Ile Phe Gln Leu Ser Glu Glu Glu Val Thr Ala Asn Met Arg Arg Gln
303      405      410      415
305 Ala Lys Ala Val Asn Tyr Gly Ile Val Tyr Gly Ile Ser Asp Tyr Gly
306      420      425      430
308 Leu Ala Gln Asn Leu Asn Ile Thr Arg Lys Glu Ala Ala Glu Phe Ile
309      435      440      445
311 Glu Arg Tyr Phe Ala Ser Phe Pro Gly Val Lys Gln Tyr Met Glu Asn
312      450      455      460
314 Ile Val Gln Glu Ala Lys Gln Lys Gly Tyr Val Thr Thr Leu Leu His
315 465      470      475      480
317 Arg Arg Arg Tyr Leu Pro Asp Ile Thr Ser Arg Asn Phe Asn Val Arg
318      485      490      495
320 Ser Phe Ala Glu Arg Thr Ala Met Asn Thr Pro Ile Gln Gly Ser Ala
321      500      505      510
323 Ala Asp Ile Ile Lys Lys Ala Met Ile Asp Leu Ala Ala Arg Leu Lys
324      515      520      525
326 Glu Glu Gln Leu Gln Ala Arg Leu Leu Leu Gln Val His Asp Glu Leu
327      530      535      540
329 Ile Leu Glu Ala Pro Lys Glu Glu Ile Glu Arg Leu Cys Glu Leu Val
330 545      550      555      560
332 Pro Glu Val Met Glu Gln Ala Val Thr Leu Arg Val Pro Leu Lys Val
333      565      570      575
335 Asp Tyr His Tyr Gly Pro Thr Trp Tyr Asp Ala Lys

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Use of n and / or Xaa has been detected in the Sequence Listing. Review the Sequence Listing to ensure a corresponding explanation is present in the <220> to <223> fields of each sequence using n or Xaa.

Use of n and / or Xaa has been detected in the Sequence Listing. Review the Sequence Listing to ensure a corresponding explanation is present in the <220> to <223> fields of each sequence using n or Xaa.

VERIFICATION SUMMARY

PATENT APPLICATION: US/09/878,131

DATE: 10/19/2001

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Input Set : A:\Lee113.app

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L:554 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:20